

## ABSTRACT

NUCLEIC ACID SEQUENCES ENCODING TYPE III TENEBRIO  
ANTIFREEZE PROTEINS AND METHODS FOR ASSAYING ACTIVITY

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A recrystallization inhibition method for determining the presence, relative concentration, and/or activity of thermal hysteresis proteins comprising: providing a proteinaceous composition in a solvent to form a test solution; flash freezing said solution; raising the temperature of the frozen solution to an appropriate annealing temperature that allows for a partial melt, while limiting heterogeneity in ice grain sizes within said solution; maintaining said frozen solution at the annealing temperature for a length of time sufficient to allow for recrystallization; monitoring the ice crystal grain size changes over time; and determining the presence of functional thermal hysteresis proteins in said solution given the retention of significantly smaller ice crystal grain sizes relative to at least one control solution.

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